

Patent claims

1. An isolated polynucleotide from coryneform bacteria,
comprising a polynucleotide sequence which codes for
5 the mikE17 gene, chosen from the group consisting of
- a) polynucleotide which is identical to the extent of at
least 70% to a polynucleotide which codes for a
polypeptide which comprises the amino acid sequence
of SEQ ID No. 2,
 - 10 b) polynucleotide which codes for a polypeptide which
comprises an amino acid sequence which is identical
to the extent of at least 70% to the amino acid
sequence of SEQ ID No. 2,
 - c) polynucleotide which is complementary to the
15 polynucleotides of a) or b), and
 - d) polynucleotide comprising at least 15 successive
nucleotides of the polynucleotide sequence of a), b)
or c) ,
- the polypeptide preferably having the activity of the
20 transcription regulator MikE17.
2. A polynucleotide as claimed in claim 1, wherein the
polynucleotide is a preferably recombinant DNA which is
capable of replication in coryneform bacteria.
3. A polynucleotide as claimed in claim 1, wherein the
25 polynucleotide is an RNA.
4. A polynucleotide as claimed in claim 2, comprising the
nucleic acid sequence as shown in SEQ ID No. 1.
5. A DNA as claimed in claim 2 which is capable of
replication, comprising
- 30 (i) the nucleotide sequence shown in SEQ ID No. 1, or

- (ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or
- (iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally
- (iv) sense mutations of neutral function in (i).
6. A DNA as claimed in claim 5 which is capable of replication,
wherein
the hybridization is carried out under a stringency corresponding to at most 2x SSC.
7. A polynucleotide sequence as claimed in claim 1, which codes for a polypeptide which comprises the amino acid sequences shown in SEQ ID No. 2.
8. A coryneform bacterium in which the mikE17 gene is attenuated, in particular eliminated.
9. The vector pCR2.1mikE17int,
9.1 the restriction map of which is reproduced in figure 1 and which
9.2 is deposited in the E.coli strain Top10/pCR2.1mikE17int under no. 14143 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ = German Collection of Microorganisms and Cell Cultures, Braunschweig) in accordance with the Budapest Treaty.
10. A process for the fermentative preparation of L-amino acids, in particular L-lysine, which comprises carrying out the following steps:

- 1 a) fermentation of the coryneform bacteria which produce
the desired L-amino acid and in which at least the
mikE17 gene or nucleotide sequences which code for it
are attenuated, in particular eliminated,
- 5 b) concentration of the L-amino acid in the medium or in
the cells of the bacteria, and
- c) isolation of the L-amino acid, the biomass and/or
constituents of the fermentation broth optionally
remaining in their entire amount or in portions in
10 the product obtained in this way.
11. A process as claimed in claim 10,
wherein
bacteria in which further genes of the biosynthesis
pathway of the desired L-amino acid are additionally
15 enhanced are employed.
12. A process as claimed in claim 10,
wherein
bacteria in which the metabolic pathways which reduce
the formation of the desired L-amino acid are at least
20 partly eliminated are employed.
13. A process as claimed in claim 10,
wherein
the expression of the polynucleotide(s) which code(s)
for the mikE17 gene is attenuated, in particular
25 eliminated.
14. A process as claimed in claim 10,
wherein
the regulatory properties of the polypeptide (enzyme
protein) for which the polynucleotide mikE17 codes are
30 reduced.
15. A process as claimed in claim 10,
wherein

for the preparation of L-amino acids, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of

- 5 15.1 the dapA gene which codes for dihydrodipicolinate synthase,
- 15.2 the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase,
- 15.3 the tpi gene which codes for triose phosphate isomerase,
- 10 15.4 the pgk gene which codes for 3-phosphoglycerate kinase,
- 15.5 the zwf gene which codes for glucose 6-phosphate dehydrogenase,
- 15 15.6 the pyc gene which codes for pyruvate carboxylase,
- 15.7 the mgo gene which codes for malate-quinone oxidoreductase,
- 15.8 the lysC gene which codes for a feed-back resistant aspartate kinase,
- 20 15.9 the lysE gene which codes for lysine export,
- 15.10 the hom gene which codes for homoserine dehydrogenase
- 25 15.11 the ilvA gene which codes for threonine dehydratase or the ilvA(Fbr) allele which codes for a feed back resistant threonine dehydratase,
- 15.12 the ilvBN gene which codes for acetohydroxy-acid synthase,

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- 15.13 the ilvD gene which codes for dihydroxy-acid dehydratase,
- 15.14 the zwal gene which codes for the Zwal protein is or are enhanced or over-expressed are fermented.
- 5 16. A process as claimed in claim 10, wherein for the preparation of L-amino acids, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of
- 10 16.1 the pck gene which codes for phosphoenol pyruvate carboxykinase,
- 16.2 the pgi gene which codes for glucose 6-phosphate isomerase,
- 16.3 the poxB gene which codes for pyruvate oxidase
- 15 16.4 the zwa2 gene which codes for the Zwa2 protein is or are attenuated, in particular eliminated, are fermented.
17. A coryneform bacterium which contains a vector which carries parts of the polynucleotide as claimed in
- 20 claim 1, but at least 15 successive nucleotides of the sequence claimed.
18. A process as claimed in one or more of the preceding claims, wherein
- 25 microorganisms of the species Corynebacterium glutamicum are employed.
19. A process for discovering RNA, cDNA and DNA in order to isolate nucleic acids, or polynucleotides or genes which code for the transcription regulator MikE17 or

have a high similarity with the sequence of the mikE17
gene,
which comprises
employing the polynucleotide comprising the
polynucleotide sequences as claimed in claims 1, 2, 3
or 4 as hybridization probes.

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